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In Vivo Pharmacokinetic Studies of Prodrugs of Ibuprofen

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Doshi, et al.: Pharmacokinetics of Prodrugs of Ibuprofen

In vivo pharmacokinetic studies of N-M annich base derivatives of ibuprofenamide as prodrugs were performed on rabbits. Ibuprofen and both the prodrugs (IBM B-M and IBM B-P) were administered orally and at different time intervals blood samples were collected and assayed for ibuprofen and ibuprofenamide by HPLC method. From the plasma concentration-time profile, (Cp)max, tmax, AUC and the time required to achieve minimum effective concentration were calculated. N-Mannich base prodrugs first get hydrolyzed to ibuprofenamide which in turn gets hydrolyzed to ibuprofen by the enzyme amidase. The (Cp)max and AUC values of IBM B-M were found to be more compared to IBM B-P. In both the cases ibuprofen started appearing after 2 h and it required minimum 4 h to get the ibuprofen in therapeutic range. Both the prodrugs released ibuprofen slowly which gave sustained effect. IBM B-M provided ibuprofen in therapeutic range for 48 h and IBM B-P for 24 h.

The non-steroidal antiinflammatory agents have major drawbacks of causing gastrointestinal ulcerogenicity. The prodrug approach was used to get a safer NSAID, where the drug containing –COOH or –OH group is converted to prodrug. The prodrugs of ibuprofen were prepared as N-Mannich base derivatives of ibuprofenamide using either morpholine or piperidine as amine component. Two prodrugs of ibuprofen were synthesized. These were, N-(morpholinomethyl) ibuprofenamide hydrochloride (IBM B-M) and N-(piperidinomethyl) ibuprofenamide hydrochloride (IBM B-P)1. The in vitro kinetic studies of the prodrugs were performed in aqueous buffers at different pH values in simulated gastric and intestinal fluids and in human plasma at 37°. The results showed that hydrolysis took place in two steps. First the N-Mannich base was hydrolyzed to ibuprofenamide which was pH-dependent and then ibuprofenamide was converted to ibuprofen which was enzymatically controlled2.

The prodrug behaves differently under in vitro and in vivo conditions because many biological factors play an important role in bioavailability and release rate of drug from prodrug during in vivo studies. The ideal way to observe the appearance of drug from prodrug is by actual studies in humans. But as the prodrugs are new drugs, it is not feasible to perform in vivo studies directly on humans3-7. Rabbit was selected
as an animal model to study the release pattern of prodrug as there are some physiological similarities of rabbits with humans. The purpose of this study is to determine the availability of drug ibuprofen and ibuprofenamide from prodrugs IBMB-M and IBMB-P; and the time required to achieve minimum effective concentration.

Four adult rabbits of either sex each weighing 3.0-3.5 kg were used in a cross over study. The protocol of the cross-over study was approved by the IAEC. Rabbits were fasted overnight but water was allowed ad libitum. Before administering the dosage form, control blood samples were obtained from marginal ear vein of all the rabbits. The ibuprofen and IBMB-P were given in suspension form (2% methylcellulose). IBMB-M was given in solution form. The drug ibuprofen (25 mg/kg) and molar equivalent of prodrug (equivalent to 25 mg/kg ibuprofen) were administered orally via Ryle’s tube (intubation tube). After drug administration, 2 ml of blood samples were collected at time intervals in the test tube containing heparin. The plasma fractions were then assayed for ibuprofen and ibuprofenamide. Graphs of plasma concentration (µg/ml) of ibuprofenamide/ibuprofen vs. time(h) were plotted (figs. 1-3).

The concentration of ibuprofen and ibuprofenamide was determined by HPLC method\(^2\). Whole blood samples were centrifuged at 1000 rpm for 15 min, the plasma was separated out using Pasteur pipette. To 0.5 ml of plasma, 2 ml of acetonitrile was added for protein precipitation, it was vortex mixed for 60 s and then centrifuged for 15 min at 2000 rpm. The supernatant was passed through C\(_{18}\) elute filter. The concentration of ibuprofen and ibuprofenamide in the filtrate was determined using HPLC. The standards were prepared daily from fresh plasma spiked with known quantities of ibuprofen and ibuprofenamide.

A solvent system acetonitrile: water (containing 1% acetic acid) 55: 45 was used at the flow rate of 1.4 ml/min. The drug was analyzed at 230 nm. Under these conditions ibuprofenamide had an elution time 4.9 min while that of ibuprofen was 7.4 min.

The in vivo studies of the prodrugs were performed on rabbits. The appearance of ibuprofenamide (IBA) and ibuprofen (IBU) from the prodrugs was observed. The prodrug as Mannich base was first hydrolyzed to ibuprofenamide, which in turn was hydrolyzed to ibuprofen. The conversion of prodrug to ibuprofenamide was pH dependent. The analysis of the plasma samples was done by HPLC method.
For the comparison purpose ibuprofen (25 mg/kg) was administered orally to the rabbits in the suspension form, and \((C_p)_{max}\) and \(t_{max}\) was determined. The \((C_p)_{max}\) was found to be 30.91 µg/ml and \(t_{max}\) was found to be 1.5 h. After 2 h, the plasma level of ibuprofen started declining. Molar equivalent of IBMB-M to 25 mg/kg of ibuprofen was administered orally to rabbits in solution form, as IBMB-M is a water-soluble prodrug.

The ibuprofenamide (IBA) started appearing after one hour and the plasma levels of IBA were found to be too low (2.16 µg/ml). After 6 h, the plasma level of IBA reached to 17.38 µg/ml and after 24 h, the plasma levels were at maximum of 20.57 µg/ml. But after that plasma level of IBA started declining; the plasma level was found to be 15.52 µg/ml after 48 h, and 5.2 µg/ml after 72 h. The \((C_p)_{max}\) was found to be 20.57 µg/ml and \(t_{max}\) was achieved within 24 h.

The ibuprofen started appearing after 2 h. After 4 h the plasma level was found to be 10.75 µg/ml. The therapeutic effective concentration of IBU is 10 µg/ml. So after 4 h, the minimum effective concentrations of ibuprofen were achieved. The plasma levels of ibuprofen were increased to 14.14 µg/ml after 6 h. After 24 h, the \((C_p)_{max}\) was achieved to 15.35 µg/ml. Even after 48 h, the ibuprofen was present in therapeutic range 11.27 µg/ml. But after 72 h, the concentration was found to be very low.

The results showed that as IBMB-M is water soluble, it did not require dissolution time. From previous kinetic studies we know that the hydrolysis rate of IBMB-M to IBA was high at acidic pH of stomach. That is why the ibuprofenamide started appearing after one hour, but the concentration was very low. There was a lag time for appearance of ibuprofen. The ibuprofen started appearing only after 2 h. Here comes the role of amidase enzyme, which is present in liver. The conversion of ibuprofenamide to ibuprofen requires amidase enzyme, so as and when ibuprofenamide was hydrolyzed from prodrug (Mannich base), it was converted to ibuprofen by amidase enzyme.

IBMB-P was given to rabbits in suspension form, so dissolution rate was also one of the factors. But from previous studies it is known that at acidic pH the dissolution rate of IBMB-P is high, so dissolution rate should not affect the absorption of prodrug.

From release rate data, it was found that IBMB-P was hydrolyzed to IBA. After 1 h, the plasma concentration of IBA was found to be 1.42 µg/ml and after six hours 24.22 µg/ml.

From the previous kinetic studies it was found that the hydrolysis rate of IBMB-P was less in acidic pH but at pH 7.4 of plasma, the hydrolysis rate was high. So in the plasma, \((C_p)_{max}\) 24.22 µg/ml of IBA was achieved after 6 h. After 24 h, the concentration was little less, 19.5 µg/ml and after 48 h it was found to be 10 µg/ml. But after 72 h, no IBA could be detected.

Ibuprofen started appearing after 2 h, as seen with IBMB-M. The peak plasma level was achieved after 6 h. The drug concentration was reached to 9.29 µg/ml after 4 h, which was very close to therapeutic concentration. After 24 h, the ibuprofen plasma level was 11.39 µg/ml, which was in therapeutic range but after 48 h; the concentration was found to be less than therapeutic concentration. After 72 h, the ibuprofen could not be detected.

The graph of plasma concentration (µg/ml) vs. time (h) was plotted. The area under curve (AUC) was calculated for both the prodrugs IBMB-M and IBMB-P. The AUC of IBMB-M was more compared to IBMB-P. In case of IBMB-M; the AUC of IBA was 1072.34 µg h/ml and for ibuprofen, 784.91 µg h/ml. And for IBMB-P, the AUC of ibuprofenamide was 935.68 µg h/ml and for ibuprofen 608.82 µg h/ml.

From these results, we can conclude that both the prodrugs release ibuprofen slowly, which gave sustained effect. Ibuprofen started appearing after 2 h and it required at least 4 h to get ibuprofen in the therapeutic range.

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Protective Effect of Tamarindus indica Linn Against Paracetamol-Induced Hepatotoxicity in Rats

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Pimple, et al.: Protective Effect of Tamarindus indica Linn

Protective effect of Tamarindus indica Linn (Caesalpiniaceae) was evaluated by intoxicating the rats with paracetamol (1 g/kg p.o.) for seven days. The aqueous extracts of different parts of Tamarindus indica such as fruits, leaves (350 mg/kg p.o.) and unroasted seeds (700 mg/kg p.o.) were administered for 9 days after the third dose of paracetamol. Biochemical estimations such as aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin and total protein were recorded on 4th and 13th day. Liver weight variation, thiopentone-induced sleeping time and histopathology were studied on 13th day. Silymarin (100 mg/kg p.o.) was used as a standard. A significant hepatoregenerative effect was observed for the aqueous extracts of tamarind leaves, fruits and unroasted seeds (p<0.05) as judged from the parameters studied.

Key words: Hepatoprotective, paracetamol, Tamarindus indica Linn, silymarin

Tamarindus indica Linn (Caesalpiniaceae) is commonly known as tamarind, (Hindi: Imli)1. It grows as a large tree and is found all over India. T indica was found to be used in jaundice and other liver complaints in folk medicine2,3. Tamarind fruit contains high amount of ascorbic acid and β-carotene, which are proved to be potent antioxidant and hepatoprotective4. The aqueous extract of leaves contain ascobic acid, β-carotene and are proved to be antilipoperoxidant, stops the peroxidation of tissue lipid and antihapatotoxic (in vitro)5. Pharmacological studies of the plant revealed that tamarind possess antibacterial, antidiabetic, antifungal, antiinflammatory, antimalarial and antioxidant activities6. Large doses of paracetamol will cause acute dose dependent necrosis in rats, mice and man7. Antioxidants can inhibit all the deleterious oxidative changes involved in paracetamol-induced toxicity8.

Fruits and unroasted seeds were procured from Pune local market. The leaves were obtained from a tree near the college campus. The plant was authenticated by Botanical Survey of India, Pune, with voucher Specimen No. BP-1. The leaves were shade dried and crushed with hand and then extracted by decoction and filtered. Fruits were cleared for any dust or foreign material and then extracted by simple maceration. Unroasted seeds were pulverized to a coarse powder and macerated in water. All the extracts were concentrated under vacuum and were stored at 0-8 throughout the study. The yield of aqueous extract for fruits leaves and an unroasted seed was 74.06% w/w, 17.55% w/w and 6.44% w/w, respectively.